## **EXHIBIT A**

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Kochhar et al. Appl. No.: 10/691,590

Conf. No.:

2216

1638

Filed:

October 24, 2003

Title:

COCOA POLYPEPTIDES AND THEIR USE IN THE PRODUCTION OF

COCOA AND CHOCOLATE FLAVOR

Art Unit:

Examiner:

: Cathy Kingdon Worley

Docket No.: 112701-536

## AFFIDAVIT UNDER 37 C.F.R. § 1.132

Sir:

I, Carl-Erik Hansen, hereby state as follows:

1. My experience and qualifications are as follows:

PhD Biochemistry. Several years of experience in food enzymology. In particular, own experience on biochemistry of cocoa fermentation and flavour generation.

- 2. I am one of the named inventors of the above-identified patent application and am therefore familiar with the inventions disclosed therein.
- 3. I have reviewed the outstanding Office Action dated June 14, 2006 pending against the above-identified patent application. As one having ordinary skill in the art, I believe that Patent Office's rejection of Claims 5-11 and 19-20 under 35 U.S.C. §112, first paragraph, is incorrect and based on a misunderstanding of the pending claims. The basis for my opinion is set forth below.
- 4. The present invention of the above-identified patent application relates, in part, to isolated or synthesized polynucleotides comprising a nucleotide sequence encoding the

Nº467

Appl. No. 10/691,590

polypeptide identified by SEQ ID NO: 1, or a fragment thereof comprising SEQ ID NO:3. For example, cocoa cells can be transformed with one or more of these polynucleotides to generate a cocoa plant from the transformed cell that is capable of producing cocoa beans with increased cocoa flavor peptides. These peptides are able contribute to stronger cocoa flavor as discussed in more detail below.

- 5. As understood by the skilled artisan, the traditional processing of cocoa beans to generate cocoa flavor requires two steps: 1) a fermentation step and 2) a roasting step. The fermentation step includes air-drying of the fermented material. During fermentation the pulp surrounding the beans is degraded by micro-organisms and the sugars contained in the pulp are mainly transformed to acids. In the course of the fermentative process, these acids slowly diffuse into the bean eventually causing an acidification of the cellular material. Furthermore, during fermentation peptides of different sizes are generated along with high levels of hydrophobic free amino acids, which are mainly attributed to the activity of specific proteinases. This specific mixture of peptides and hydrophobic amino acids is thought to be cocoa-specific flavor precursors.
- 6. A number of different types of enzymes, such as an aspartic endoproteinase, a cysteine endoproteinase or a carboxypeptidase have been found to participate in these degradative reactions leading to the formation of the cocoa flavor peptide/amino acid precursor pool. During the second step of cocoa flavor production (i.e. the roasting step), oligopeptides and amino acids generated during the fermentation stage are subjected to a Maillard reaction in the presence of reducing sugars in the mixture thereby yielding substances understood to be responsible for the typical cocoa flavor.
- 7. There have been attempts to artificially produce cocoa flavor in the past, such as, by subjecting acetone dried powder prepared from unfermented ripe cocoa beans to autolysis at a pH of 5.2 followed by roasting in the presence of reducing sugars. It was taught that under these conditions preferentially free hydrophobic amino acids and hydrophilic peptides would be generated. The peptide pattern obtained from this process was found to be similar to that of

Nº467

Appl. No. 10/691,590

extracts from fermented cocoa beans. In studies, Leu, Ala, Phe and Val were found to be the predominant amino acids liberated in fermented beans or autolysis. In contrast to these findings, no cocoa-specific flavor could be detected when the above powder was subjected to autolysis at a pH of 3.5. Few free amino acids were found in the by-product of the autolysis, but there were a large number of hydrophobic peptides formed. Synthetic mixtures of free amino acids having a composition which resembles that found in fermented beans also have been found not to produce the cocoa flavor desired. These findings indicate that both the peptides and the amino acids are important in producing cocoa flavor.

- 8. To date, four major proteins with an apparent molecular weight of 14.5, 21, 31 and 47 kDa, have been identified before fermentation in cocoa beans. These proteins are thought to give rise to the peptide/amino acid pool responsible for producing the cocoa flavor. The present invention provides, in part, additional proteins responsible for providing a stronger cocoa flavor.
- 9. As one having ordinary skill in the art, I believe that the polypeptides according to the present invention can be used in the manufacture of a stronger cocoa flavor. The polypeptides may be added to a fermentation mixture of cocoa beans to provide a higher amount of the polypeptides for degradation. In addition, cocoa plants can be modified by recombinant means and contain a high number of copies of nucleotide sequences encoding the polypeptides of the present invention. These modified cocoa plants will inherently contain a higher concentration of the polypeptides and eventually will result in the production of a stronger cocoa flavor after conventional processing.
- 10. For all the foregoing reasons, as one having ordinary skill in the art, I believe that it is within the capabilities of one having ordinary skill in the art to make and use the claimed invention. I believe that there is adequate disclosure of transgenic cocoa beans that express the polypeptides or fragments thereof in the present specification. Transformed cocoa plants in accordance with the present invention can be made by techniques well known in the art and will contain a higher concentration of the polypeptides thereby resulting in cocoa beans having a

Appl. No. 10/691,590

stronger cocoa flavor after processing. Methods of manufacturing cocoa flavor from these transformed cocoa plants and their cocoa beans are also well known. As a result, the skilled artisan can readily determine the enhanced cocoa flavor from the transformed cocoa plants after processing of the transgenic cocoa beans.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

Date: 2006-09-12

Name: Carl-Erik Hausen